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Prevention of Lactogenic *Toxocara cati* Infections in Kittens by Application of an Emodepside/Praziquantel Spot-on (Profender®) to the Pregnant Queen

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Abstract

This study aimed to evaluate the efficacy of an emodepside 2.1 % (w/v)/praziquantel 8.6 % (w/v) topical solution (Profender® spot-on for cats) in the prevention of lactogenic *Toxocara cati* infections. A controlled test was performed with two groups of 8 cats with confirmed pregnancy. All cats were infected with daily doses of 2000 *T. cati* eggs for 10 consecutive days starting 50 days post conception to produce an acute infection. Treatment was performed 60 days post conception. Queens in the treatment group received the emodepside/praziquantel solution at the minimum therapeutic dose (3 mg/kg emodepside and 12 mg/kg praziquantel), while the control group was treated with a placebo spot-on. Efficacy was evaluated 56 days post partum by necropsy of one randomly selected kitten of each litter and comparison of the worm burdens between the study groups. Additionally the necropsy results were supported by quantification of worms expelled with the faeces after deworming of the remaining kittens and all queens. The treatment in late pregnancy resulted in an efficacy of 98.7 % ($p < 0.0001$). All necropsied control kittens were infected (geometric mean 30.6). Seven of 8 kittens from treated mothers were free of *T. cati* (geometric mean 0.4). Worm counts after deworming reflected the results obtained at necropsy. No side effects of the treatment were observed. It is concluded that treatment with an emodepside/praziquantel spot-on solution during late pregnancy effectively prevents lactogenic transmission of *T. cati* to the offspring. The study design facilitated the generation of reliable data, while at the same time a minimum number of animals was sacrificed.

Introduction

Vertical transmission, i.e. transmission of developmental stages from the infected mother to the offspring either prenatally or by the transmammary pathway, is a major component in the life cycles of several species of parasitic helminths (Miller 1981; Shoop 1991). It occurs predominantly in nematodes, but some trematode species such as *Alaria* spp. (Shoop and Corkum 1983, 1984; Foster et al. 2009) and cestodes, e.g. *Mesocestoides corti* (Conn and Etges 1983), have also been reported to be vertically transmitted.

The biological significance of these transmission routes has been demonstrated for many species of the genus *Strongyloides* (Lyons et al. 1973; Stewart et al. 1976; Wilson et al. 1978; Nwaorgu and Onyali 1990), for *Toxocara* spp. in dogs (Burke and Roberson 1985a,b), cats (Swerczek et al. 1971) and bovines (Roberts et al. 1990) and for the hookworm species *Ancylostoma caninum* (Burke and Roberson 1985a,b) and *Uncinaria lucasi* (Olsen and Lyons 1965). The lactogenic route is more common than the transuterine pathway and the pattern of transmission varies between species (Shoop 1991). In several species the transmission of hypobiotic larvae that are reactivated during late pregnancy is a pathway of great biological importance. These larvae invade the foetuses directly or accumulate in the mammary glands to be transmitted via the milk after parturition. While both ways of vertical transmission occur in bitches chronically infected with *Toxocara canis*, *Toxocara cati* has only been shown to be lactogenically transmitted after acute infection during late pregnancy (Coati et al. 2004) (Fig. 1).

Toxocara cati is the most common gastrointestinal helminth of cats worldwide. In stray cats and fostered cats prevalences of up to 44 % are found (Becker et al. 2012; summarised by Wolken et al. 2009). But also privately owned cats are regularly infected. Especially young kittens are at high risk of infection, which can be explained by the vertical transmission of this parasite. In a study by Barutzki and Schaper (2013) Infection rates of up to 11.4 % were found in kittens younger than 3 month. Various attempts at anthelmintic treatment of the dam to protect the offspring from vertical infections have been made in the past. These studies mainly focused on dogs for the control of neonatal infections with *T. canis* or *A. caninum* (see Krämer et al. 2006 and 2009 for an overview of treatment protocols) and on *Strongyloides* spp. in pigs (Barth et al. 1996; Drag et al. 1998) and horses (Ludwig et al. 1983). Concerning *T. cati*, a study by Wolken et al. (2009) used faecal egg counts (FEC) to evaluate the efficacy of an emodepside/praziquantel spot-on treatment in the prevention of lactogenic infections. Egg shedding was completely prevented in the offspring (n=10) of 4 queens that had received a single treatment during late pregnancy. The aim of the study presented here was to verify the hypothesis that a single treatment with emodepside/praziquantel spot-on treatment will prevent lactogenic transmission of *T. cati* in a controlled test using necropsy worm counts as an efficacy criterion as recommended by anthelmintic guidelines.

Materials and methods

The study was performed as a monocentric, placebo-controlled, randomised and blinded efficacy study. As no detailed guideline on vertical transmission studies exists, the study was performed in accordance with VICH guideline 9 “Good Clinical Practice” (June 2000). Furthermore the recommendations given in VICH Guideline 7 “Efficacy of anthelmintics: general requirements” (November 2000), VICH Guideline 20 “Efficacy of anthelmintics: Specific Recommendations for Felines” (June 2001) and the WAAVP guidelines for evaluating the efficacy of anthelmintics for dogs and cats (Jacobs et al. 1994) were followed whenever possible. The general study design was based on experience published in the scientific literature (Coati et al. 2004; Wolken et al. 2009).

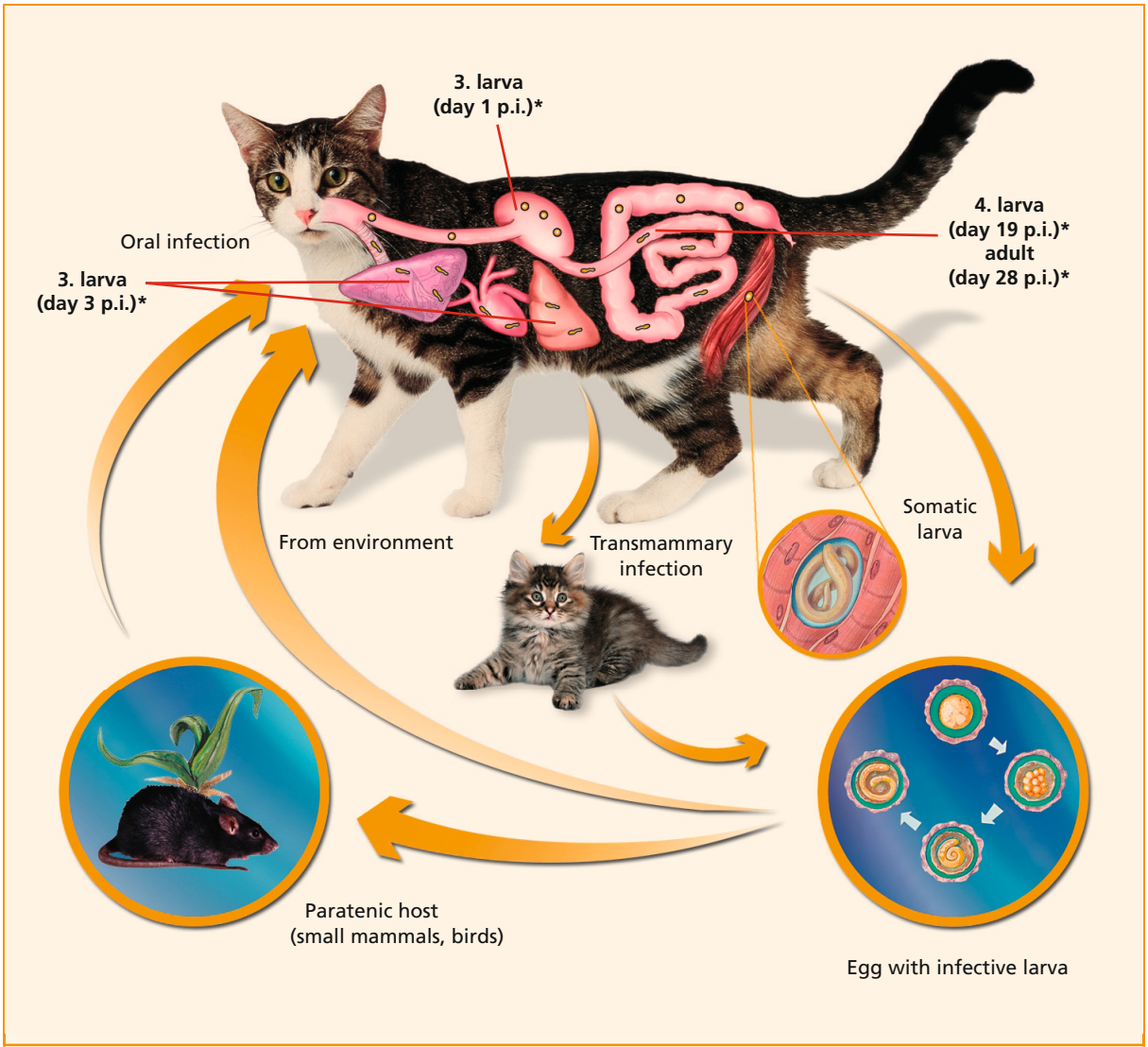

Fig. 1: Life cycle of *Toxocara cati* including location and first time point of appearance of the developing larval stages (*occurrence of larvae in the respective organ according to Sprent 1956)

Study animals

Sixteen female domestic short-hair cats, aged between 1.5 and 6.5 years and originating from the cat colony of the Institute for Parasitology, University of Veterinary Medicine Hannover, were included in the study. The animal experiment was approved by the ethics commission of the State Office for Consumer Protection and Food Safety of the German federal state Lower Saxony. Due to the fact that it was not possible to have 16 pregnant queens at a time, the study was conducted during various breeding seasons between March 2012 and November 2013. Mating, parturition and rearing proceeded under natural conditions with the exception of one queen where a caesarean section was necessary. Whenever a cat showed signs of oestrus, it was brought together with the tomcat. In order to have a precise mating date the couple stayed together for no longer than one day. Pregnancy was confirmed by ultrasonic examinations approximately 3 weeks after mating. The number of foetuses was determined and later used for

randomization (see section "allocation and treatment" below). Only queens with more than one live fetus at the ultrasound examination were included in the study. Queens and their litters were individually housed in tiled indoor pens with a floor space of 2.1 to 7.7 m^2 and a height of approximately 2.3 m. Shortly before expected parturition a whelping area was provided. The cats received amusement toys for environmental enrichment as well as daily grooming and playing time with the animal attendants. The pens were cleaned with 60 – 80 °C hot water and disinfected with cresole (Neopredisan®, Menno Chemie-Vertrieb GmbH) on a regular basis. The cats had unlimited access to water and were fed standard diets (Pediatric Queen®, Pediatric Weaning®, Royal Canin). For collection of individual faeces at study end the queens and kittens were housed singly in plastic coated wire cages equipped with a raised resting area, litter tray and toys for enrichment. Cats were handled with due regard to their well-being.

Health observations

General health observations were conducted daily. Physical examinations of the queens were performed twice between mating and the first experimental infection and on the day of treatment. Only healthy cats were included in the study. All queens and kittens underwent a physical examination within 48 hours after parturition and at study end (56 days after parturition). Clinical assessments were performed prior to treatment and at 3 and 24 hours post treatment. Body weights in queens were determined on study day 0 for dosing, in kittens within 48 hours after birth and in all cats at study end.

Experimental infections

Cats were infected with embryonated *T. cati* eggs recovered from the faeces of an experimentally infected cat. The strain was maintained at the Institute for Parasitology, University of Veterinary Medicine Hannover and originated from a field isolate identified in Germany in 2010. The eggs were microscopically checked for viability of larvae, suspended in tap water and applied orally with a syringe. Starting 50 days after conception each queen was infected daily with approximately 2,000 infective *T. cati* eggs for 10 consecutive days (study day -10 to -1) to produce an acute infection.

Allocation and treatment

In order to have an equal distribution of kittens in the treatment groups, the queens were allocated according to the results of the ultrasonic examination. Based on the expected litter size the queens were assigned to one of 2 random draws, one for an expected litter size of 2 to 4 kittens and one for a litter size equal to or greater than 5. The queens were allocated in the order they had their ultrasound examination. Treatment was performed on study day 0 (one day after the last experimental infection). Queens treated with the investigational veterinary product (IVP) (Profender® spot-on for cats) were dosed with 0.14 mL of the spot-on formulation per kilogram (kg) body weight, which corresponded to the recommended minimum therapeutic dose of 3 mg/kg emodepside and 12 mg/kg praziquantel. Cats assigned to the control group were treated with a placebo spot-on formulation, using the same amount of 0.14 mL per kg body weight to mimic the appearance of the IVP. Treatments were performed according to the label instructions of the manufacturer.

Faecal examinations

Three faecal egg counts (FEC) were performed during the week prior to the first experimental infection to demonstrate the parasitological status of the cats. For study inclusion these counts had to be negative for nematode eggs. To monitor the onset of patency, FECs were performed 3 times per week starting 13 days post partum (p.p.) in queens and 21 days p.p. in kittens. Due to the mother's intensive grooming activities it was difficult to obtain individual faecal samples from the kittens. Therefore, efforts were made to receive at least a faecal smear directly from the rectum (by the use

of a cotton bud) to demonstrate the parasitological status of the individual kitten. A few pooled faecal samples from the whole litter were found by chance and examined additionally. A final individual FEC was performed at study end to demonstrate the success of the final deworming. FECs were performed using a modified McMaster technique (Wetzel 1951).

Necropsy

56 days p.p. one kitten from each litter was randomly selected and euthanised for necropsy. An exception from random selection was made when, due to the premature death of the siblings, only one kitten was left on the day of necropsy and in one case where a kitten in the control group was affected by a congenital deformation of the tarsal joint. For ethical reasons this kitten was selected for necropsy.

At necropsy the digestive tract from the beginning of the stomach to the rectum was removed and the intestinal content and the results of several mucosal strippings were washed over sieves with mesh size of 50 µm (stomach and small intestine) or 100 µm (large intestine). All contents were analysed under a stereo microscope and the recovered parasites were counted and identified for species and developmental stage.

Deworming and collection of excreted worms

On day 56 p.p. all queens and those kittens that were not selected for necropsy were dewormed with pyrantel embonate plus praziquantel (Drontal® tablets for cats, Bayer) at the dose recommended by the manufacturer. All faeces were collected for at least 3 days and examined macroscopically for excreted worms.

Efficacy determination and statistical analysis

The adequacy of infection was determined according to VICH guidelines 7 and 20. These require a minimum of 6 animals in the control group with at least 5 worms each. Additionally the lower limit of the 95% confidence interval was required to be greater than 10% of the central tendency (geometric mean as all counts were > 0).

Percentage efficacy of the treatment was calculated according to VICH guideline 7 and the WAAVP guideline for evaluating the efficacy of anthelmintics for dogs and cats (Jacobs et al. 1994) as follows:

% Effectiveness (reduction) = (N1 – N2)/N1 x 100

N1: geometric mean (GM) of *T. cati* counts in kittens from placebo treated queens
N2: geometric mean (GM) of *T. cati* counts in kittens from Profender® treated queens

For these calculations the sum of all *T. cati* stages that were found at necropsy (larvae, premature and adult stages) was used. Due to the presence of worm count values of "0", all counts were modified by adding 1 prior to log transformation and subtracting 1 from the antilog value. The non-parametric Wilcoxon rank sum test (two tailed, $\alpha = 0.05$) was used to test for a treatment group (emodepside/praziquantel vs. placebo) effect. Additionally the medical relevance of the difference between the groups was quantified using the Mann-Whitney superiority measure (MW) and its two-sided 95% confidence interval as the corresponding effect size. The analyses were performed using the software Testimate Version 6.5 (IDV Gauting).

Results

The duration of pregnancy was within physiological limits (Linde-Forsberg and Eneroth 1998) and varied between 63 and 68 days in the IVP-treated group and between 64 and 69 days in the control group. Due to these variations, the implemented study design resulted in a treatment time point between 3 and 9 days pre partum. A total of 54 kittens (29 in the control group, 25 in the IVP treated group) were borne during the study. One litter had

to be delivered by caesarean section after the still birth of one kitten and subsequent absence of contractions. Rearing of this litter proceeded under natural conditions. Six kittens in the control group and 2 in the IVP-treated group were born dead or died during the study. It became apparent that infections with the Feline Leukemia Virus (FeLV) were present in the study population. Two kittens in a control litter showed deformation of the tarsal joints at birth. While one kitten recovered and showed normal development during the study, the other was still affected at study end. All other kittens and the queens stayed clinically healthy throughout the study. No negative side effects of the treatment were detected.

The acute infection during late pregnancy produced patent infections in 7 of 8 queens in the control group. Onset of patency was between 43 and 69 days after the first experimental infection. Requirements for an adequate infection in the control group were fulfilled. All 8 necropsied kittens harboured *T. cati* (range: 7–72, GM = 30.6) (Tab. 1). Seven kittens from the IVP-treated queens were negative at necropsy. One kitten harboured a total of 12 *T. cati* in the intestine (GM = 0.4). The treatment resulted in an efficacy of 98.7 %. The Profender®-treated group was highly statistically significantly superior to the control group ($p < 0.0001$, MW-statistic = 0.9766).

Seven queens in the control group excreted worms after deworming (range 1–202). The queen that was constantly negative at the FEC examinations (litter ID 15) also did not excrete worms. In contrast, the necropsied kitten from this queen, which was the only kitten that was left in this litter at necropsy, showed a high worm burden (55), confirming successful vertical transmission in this litter too. In 13 of 15 dewormed control kittens excreted worms could be found in the faeces (range 1 – 39). In one litter (litter ID 9) 2 control kittens showed no worm excretion. However, a third kitten of the same litter excreted 13 *T. cati* stages. Two of 15 kittens from Profender-treated queens each excreted 3 worms after deworming. These 2 kittens belonged to the same litter as the kitten that was positive at necropsy (litter ID 6) (Tab. 1).

Discussion

The aim of this study was to verify preliminary data from a study by Wolken et al. (2009) on the efficacy of an emodepside/praziquantel spot-on formulation (Profender® for cats) in the prevention of vertical transmission of *T. cati* in cats. As suggested by the anthelmintic guidelines, a controlled test was used. No detailed recommendations existed regarding the design for a vertical transmission study, therefore the general requirements of the anthelmintic guidelines were implemented together with the standards of GCP and current scientific knowledge.

To date it has been shown that lactogenic transmission of *T. cati* larvae occurs only after acute infection of the queen during late pregnancy and not during chronic infections (Coati et al. 2004). To mimic this situation, the infection protocol used by Coati et al. (2004) and Wolken et al. (2009) was successfully adopted in this study. Vertical infection was proven in 21 of 23 kittens in the control group. Two kittens that did not excrete *T. cati* stages after deworming belonged to a litter with 4 kittens. The necropsied kitten, the fourth kitten and the mother were infected, confirming successful vertical transmission in all 8 control litters. Due to the scarce literature dealing with vertical transmission of *T. cati*, it is almost impossible to assess the biological variability that can be expected in terms of transmission rates. The only 2 studies reporting detailed data showed transmission rates of 100 % in kittens necropsied 8 to 22 days p.p. (Coati et al. 2004) or 5 to 20 days p.p. (Swerczek et al. 1971). Altogether data from these two publications represent the results of 8 litters with a total of 21 kittens that were allowed to nurse long enough for transmission to take place. Three kittens examined 1 or 3 days p.p. by Coati et al. (2004) were not infected.

One queen in the control group stayed coproscopically negative throughout the study. Her kitten, however, was positive at necropsy, providing proof of a technically correct experimental infection of the mother. This queen was the same one that needed a caesarean section. Whether the cause of the absent colonization of the queen's intestine is just biological variance or the result of a negative influence of the anaesthesia can only be speculated. Coati et al. (2004) performed a caesarean section on 4 chronically *T. cati*-infected queens with positive FECs to evaluate the possibility of prenatal infections. All queens stayed coproscopically positive throughout the observation period of several weeks after surgery. Therefore the anaesthesia had no negative effect in this case. It has to be considered, however, that different anaesthetics may have been used and that the worms in the study by Coati et al. (2004) were adult at the time of caesarean section. In the study presented here the coproscopically negative queen was infected 13 to 4 days prior to surgery. According to Sprent (1956), larvae first appear in the lungs on day 3 post infection, and reach the intestine by day 19 post infection. Starting day 10 p.i. larvae can be found in the muscle tissue. It thus seems possible that tissue migrating larvae from the first days of infection had already reached the mammary glands on the day of caesarean section, and were not affected by the anaesthetic while larvae on the route of tracheal migration were exposed to high concentrations. Nevertheless, an influence of general anaesthesia on the worm burden has to our knowledge not been reported before in veterinary medicine.

Table 1: Numbers of *T. cati* stages at necropsy and after deworming

Study group	Litter ID	Worm counts at necropsy				Worm counts after deworming			
		Individual	Total	Geometric mean	Efficacy	Kittens		Queens	
						Individual	Total	Individual	Total
Profender® Spot-on	1	0	12	0.4	98.7 %	0/0	6	0	0
	2	0				0/0		0	
	3	0				0		0	
	4	0				0		0	
	5	0				0		0	
	6	12				3/0/0/0/3		0	
	7	0				0/0/0		0	
	8	0				–		0	
Placebo	9	32	305	30.6	–	13/0/0	185	33	558
	10	50				17/26		202	
	11	72				–		113	
	12	7				6/4		37	
	13	12				1/24/13		1	
	14	34				2/39/14		167	
	15	55				–		0	
	16	43				1/25		5	

The treatment was well tolerated by the queens and no negative side effects were seen in the kittens. Still births and premature deaths in both study groups were most likely due to an infection with the Feline Leukemia Virus (FeLV), which is associated with abortions, still birth and weak kittens ("Fading Kitten Syndrom") (Hartmann 2012).

Necropsy worm counts in kittens from Profender®-treated queens were reduced by 98.7 % compared to kittens from queens that received the placebo solution. Seven of 8 kittens in the treated group were negative at necropsy. For ethical reasons only one kitten per litter was euthanised and examined for stages of *T. cati*. To take account of biological variations, and therefore to minimize the risk of selecting a negative kitten for necropsy while other kittens of the litter were positive, the results obtained by necropsy were supported by deworming the remaining kittens and all queens and collecting the excreted worms. The results obtained by deworming reflected those at necropsy. One kitten from a treated queen was positive at necropsy and belonged to a litter with 6 kittens. Two further kittens from this litter were positive at deworming, the other 3 and the queen were negative. All other queens and kittens in the treated group were also negative. When expelled worms after deworming are used to determine worm burdens like it is done in a critical test, no drugs may be used that destroy the parasite's body (VICH GL7). The use of pyrantel and the fact that ascarids have a thick hypodermis and cuticula, and are thus not easily digested, allowed reliable data to be generated by this method.

None of the kittens developed a patent *T. cati* infection. This can be explained by the fact that mainly pre-adult stages and only single adults were found in the kittens. Information in the literature on the prepatent period after lactogenic transmission of *T. cati* is scarce. Kittens of one litter in the study by Coati et al. (2004) became patent on days 44, 53 and 55 after birth. In the study by Wolken et al. (2009) one litter in the control group became coproscopically positive 36 days after birth and the other on day 50 after birth. A different *T. cati* strain was used in the present study that might have exhibited different biological properties. Biological variations have to be considered in every study with experimental infections. The results of this study demonstrate that the study design was able to cope with these variations, while at the same time a minimum number of animals were sacrificed. But the fact, that not all kittens in the control group were infected also shows that supportive data are important to exclude false negative results in a vertical transmission study design that avoids euthanasia of the whole offspring.

It can be concluded, that a single treatment of queens with an emodepside/praziquantel topical solution (Profender® spot-on) in late pregnancy is safe and effectively prevents the offspring from becoming infected with *T. cati* via nursing.

Ethical standards

The study was performed in compliance with current national laws and regulations.

Funding

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Conflict of interest

At the time the study was conducted, Claudia Böhm was employed by the University of Veterinary Medicine, Hannover, and is currently an employee of Bayer Animal Health GmbH. Gabriele Petry and Roland Schaper are employees of Bayer Animal Health GmbH. Sonja Wolken was at the time of study initiation employed by the University of Veterinary Medicine, Hannover, and is currently a self-employed consultant. Christina Strube is employed by the University of Veterinary Medicine, Hannover.

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